1997

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 070464-R; ELEXA (0.9500% Chitosan), Broad Spectrum Biofungicide; Product Chemistry, Acute Toxicity Studies and Ecological Effects Waiver Request; MRID Nos. 441858-01 through -14 (Chemical #128930; Case No. 061013; Submission #S521231; DP Barcode D234872).

FROM: Carol E. Glasgow, Ph.D.
Biological Pesticide Branch
Biopesticides and Pollution Prevention Division (7511W)

THROUGH: Roy D. Sjoblad, Ph.D., Chief
Biological Pesticide Branch
Biopesticides and Pollution Prevention Division (7511W)

TO: Rita Kumar
Regulatory Action Leader
Biochemical Pesticide Branch
Biopesticides and Pollution Prevention Division (7511W)

Action Requested:

On December 23, 1996, Edgar R. Butts, Ph.D., acting as Regulatory Consultant and Agent for Agricultural Glycosystems, Inc. (AGI), submitted several studies and related data to fulfill requirements for the registration of ELEXA. These included: 151-10, 151-11, 151-12, 151-13, 151-16, 151-15, 151-17, 152-10, 152-11, 152-12, 152-13, 152-14, 152-15, 152-16 (MRID No., 441858-01 through -14). A waiver request for ecological effects data was also included. Wildlife International, Ltd. performed testing on MRID numbers 441858-04, -06 and -07 and IIT Research Institute (ITTRI) conducted 441858-08 through -12. The other MRIDs completed by the manufacturer.

Dr. Butts summarized decisions from a June 11, 1996 meeting with EPA in a letter to EPA dated July 1, 1996. In this letter, Dr. Butts requested waivers for certain additional toxicity tests and the ecological effects tests. These were as follows: Toxicity Tests -- 152-17, 152-18, 152-20, 152-23; Ecological Effects Tests: 154-6, 154-7, 154-8, 154-9, 154-10, 154-11 plus toxicity to estuarine and

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marine fish, mollusks and shrimp, and acute toxicity to the honeybee (no guideline numbers provided).

On June 11, 1997, I spoke with Bruce Jaeger of Stewart Pesticide Associates, Inc., AGI's new agent for this product, about information missing from the application packet. The active ingredient is not manufactured by AGI but purchased, and on p.4 of 151-12, it was stated that a letter from the manufacturer on the purity of the compound was included. Further, the cover letter stated a data matrix of research was included that provided justifications for the waiver requests. These were missing. Moreover, each of the toxicity studies performed by IITRI stated the material was supplied in a bucket and kept at room temperature from July 2nd through the 8th. Then the sponsor directed the laboratory to refrigerate the product. The sponsor also requested the lab to filter the product through a 0.45 micron filter before testing. There was no justification presented in the packet for this unusual request.

On the 16th of the month, I again spoke to Mr. Jaeger about the letter of purity for the active. He stated the manufacturer was granted an exemption of tolerance and the application for exemption included purity information. However, Mr. Jaeger was not aware the CSF listed two sources for the active, and I told him EPA would need purity information for both.

Ms. Kumar of the Biological Pesticide Branch (BPB) received a letter from Mr. Jaeger detailing AGI's response dated 20 June 1997 and, at the same time, OPP received the thermal stability data on **ELEXA**, MRID 442923-01. Mr. Jaeger listed the following:

- "A thermal stability study conducted by Wildlife International LTD in accordance with OPPTS 830.6313 ... demonstrated that there was no degradation in the stability or composition of ELEXA for up to 14 days at 55°C and 20°C with overall chitosan content ranging from 1.14 to 1.18% on day 0, and from 1.26 to 1.13% on day 14, respectively."

- "The sponsor stated that this (filtration) was done at his request because filtration is part of the manufacturing process which is now done prior to packaging. At the time this material was shipped, filtering was not a step conducted by the plant. However, the materials eliminated by this filtration step are by-products of the extraction of the active ingredient (i.e. crab shell fragments) which are removed because they clog spray equipment nozzles and interfere with dispersement of product in the field."

- "I have appended a copy of the version (data matrix) which was submitted to EPA by E.R. Butts International, Inc. on December 23, 1996."

- AGI has "...decided to delete [redacted] from the CSF as a supplier of chitosan since they have not been used for some time [redacted] is their sole supplier of chitosan."

CONCLUSIONS: My comments regarding Mr. Jaeger's statements are as follows:

- The thermal stability study satisfies the requirement for storage stability

- The sponsor's reasons for filtration justify the change
- The data matrix provides the needed justification for the requested waivers
- The new CSF, plus the information from the [redacted] request on the purity of chitosan supplied for [redacted] provides the needed data for this requirement.

BPB considers the material, both chemistry and toxicity data supplied by AGI, sufficient to approve the application.

TOXICITY PROFILE

Acute oral toxicity	IV	Acceptable
Acute dermal toxicity	III	Acceptable
Acute inhalation toxicity	IV	Acceptable
Primary eye irritation	IV	Acceptable
Primary dermal irritation	IV	Acceptable
Dermal sensitization	No	Acceptable

LABELING: The signal word is "Caution" from the category III rating for acute dermal toxicity. The label however, is inappropriate in calling the product a biofungicide. This would amount to a significant new use, and per his telephone conversation with Rita Kumar, Mr. Jaeger will be altering the label to avoid the need for a RED. The company will label **ELEXA** a plant growth regulator. AGI presently states on the label under **GENERAL** that the sprayed product will help plants fight off the llisted diseases by enhancing the plant's natural defense system. AGI will now delete the claim this product "controls" certain plant diseases.

The current label includes excessive precautionary statements of practical treatment for human health, and worker and wildlife protection warnings which, under the circumstances, are superfluous. Below is appropriate wording from the Label Review System:

SIGNAL WORD: CAUTION

PRECAUTIONARY STATEMENTS:

Harmful if absorbed through skin. Avoid contact with eyes, skin or clothing. Wash thoroughly with soap and water after handling.

STATEMENT OF PRACTICAL TREATMENT (SOPT):

IF ON SKIN: Wash with plenty of soap and water. Get medical attention. For Category III, add "if symptoms persist."

The reviews of data by BPB are summarized below.

Study Summaries:

PRODUCT CHEMISTRY

Guideline No. 151-10: Product identity and disclosure of ingredients (MRID 441858-01)

ELEXA contains 0.95% chitosan (poly-D-glucosamine), a naturally-occurring compound as its active ingredient, and 95.05% inert ingredients. This product is to be used to control or suppress *Alteria alternata, Botrytis cinerea, Colletotrichum spp., Fusarium spp., Pythium spp., Phytophthora infestans, Piricularia oryzae, Rhizoctonia spp.*, and various species of Downy and Powdery mildew.

The following table summarizes information submitted by the registrant regarding the active ingredient.

Chemical Name:	Poly-D-glucosamine
CAS Registry No.:	9012-76-4
Synonyms:	Chitosan
Chemical Family:	Oligosaccharide
Source of Biochemical:	Crustacean exoskeleton
Mode of Action:	Plant growth regulator

Chitosan is a naturally-occurring compound produced from chitin extracts of crustacean shells (e.g., crab, shrimp and lobster). In 1995 EPA approved an exemption for chitosan from the requirement for tolerance on raw agricultural commodities. Other common applications of poly-D-glucosamine are as a soil amendment (fertilizer), and FDA has approved certain chitin-based products in foods as hypocholesterolemic agents, as dietary fiber in low-calorie diets and as agents to increase the specific loaf volume of bread.

An acceptable confidential statement of formula was submitted by the registrant, except for omission of 151-10(c)(2)(viii), the structural formula of the inert. However, as the inert is chemically non-toxic, BPB does not consider this omission important.

BPB's Comment: The submitted data on the product identity satisfy the requirements of 40 CFR 158.690.

Guideline No. 151-11: Manufacturing process (MRID 441858-02)

In Confidential Appendix

Guideline No. 151-12: Discussion on the formation of unintentional ingredients (MRID 441858-03)

In Confidential Appendix

Guideline No. 151-13: Analysis of samples (MRID 441858-04)

In Confidential Appendix

Guideline No. 151-15: Certification of ingredient limits (MRID 441858-05)

In Confidential Appendix

Guideline No. 151-16: Analytical methods for certified limits (MRID 441858-04)

In Confidential Appendix

Guideline No. 151-17: Physical and Chemical Characteristics (MRIDs 441858-06 and -07)

The registrant submitted information on the physical and chemical characteristics of the formulated end-use product which are summarized below:

Study Type	Characteristic	Source
Color	Colorless	MRID 441858-06
Physical State	Liquid at 20°C - small suspended white particles	MRID 441858-06
Odor	Halide - very faint musty, moldy smell	MRID 441858-06
Density	1.00 g/ml at 20°C	MRID 441858-06
(Specific Gravity)	(1.003)	MRID 441858-06
pH	5.34 at room temperature	MRID 441858-06
Storage Stability	Thermally stable for 2 weeks at 20°C and 55°C or in sunlight; no endothermic or exothermic reactions with aluminum, iron or tin	MRID 442923-01
Viscosity	100-1200 centipoises minimum (Brookfield 1% in 1% acetic acid)	MRID 431732-00 (Reg. No. 67883-1)
Corrosion Characteristics	0 mils/yr of HDPE at 20°C	MRID 441858-07

BPB's Comment: The submitted information on chemical and physical characteristics satisfies the requirement of 40 CFR 158.690.

PRODUCT TOXICOLOGY

Guideline No. 152-10: Acute Oral Toxicity Study in Rats (MRID 441858-08) A single, limit dose (5,000 mg/kg) gavage dose of the test compound was tested in male and female rats. All treated animals gained weight during the study, with no clinical symptoms observed. The LD_{50} of ELEXA was determined to be greater than 5,000 mg/kg. Coregraded: Acceptable; Toxicity Category IV.

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Guideline No. 152-11: Acute Dermal Toxicity Study in Rabbits (MRID 441858-09) A single, limit-dose (2,000 mg/kg) application of the test compound was tested in male and female rabbits. All treated animals gained weight during the study, and slight erythema observed in 4 males, and 2 females at removal of coverings, cleared by day 2. The LD_{50} of ELEXA was determined to be greater than 2,000 mg/kg. Coregraded: Acceptable; Toxicity Category III.

Guideline No. 152-12: Acute Inhalation Toxicity Study in Rats (MRID 441858-10) A single maximum achievable dose (>3.6 mg/l) concentration of the test material was tested in male and female rats. All treated animals gained weight during the study, and no clinical signs observed. Uterine horns dilatated in 2 females at necropsy. The LC_{50} of ELEXA was determined to be greater than the tested concentration (>3.6 mg/l). Coregraded: Acceptable; Toxicity Category IV.

Guideline No. 152-13: Primary Eye Irritation Study in Rabbits (MRID 441858-11) A single (0.5 ml) dose applied to male and female rabbit's eyes. Grade 1 conjunctivitis seen in 5/6 rabbits (hyperemia) and also discharge in 1 of the hyperemic animals at 1 hour reading and clear by 24 hours. Coregraded: Acceptable; Toxicity Category IV.

Guideline No. 152-14: Primary Dermal Irritation Study in Rabbits (MRID 441858-12) A single (0.5 ml) dose applied to site on rabbit skin. No erythema or edema observed at any reading. Coregraded: Acceptable; Toxicity Category IV.

Guideline No. 152-15: Delayed Contact Hypersensitivity (Dermal Sensitization) in Guinea Pigs (MRID 441858-13) When a modified Buehler method (Ritz and Buehler, 1980) is used, the test compound does not appear to be a dermal sensitizer in male Hartley guinea pigs, following 3 inductions (100%) and challenge (100%). No indication of dermal erythema after challenge or induction. Coregraded: Acceptable; Not a dermal sensitizer.

Guideline No. 152-16: Hypersensitivity Incidents Report - ELEXA (MRID 441858-14) No incidents reported.

Guidelines No. 152-17: Studies to detect genotoxicity; 152-18: Cellular immune response; 152-20: 90-Day feeding; 152-23: Teratogenicity The manufacturer of this product has requested waivers for the toxicologic studies listed here. AGI believes these are justified based on the low concentration of chitosan in ELEXA, its low toxicity and its widespread natural occurrence in the environment. BPB grants this request.

154-6: Acute avian oral toxicity - Bobwhite; 154-7: Acute avian dietary toxicity - Mallard; 154-8: Freshwater fish toxicity - Rainbow trout; 154-9: Freshwater invertebrate toxicity - *Daphnia*; 154-10: Nontarget plant testing - End use product; 154-11: Nontarget insect testing - End use product; Acute toxicity - Honeybee The manufacturer of this product has requested waivers for the ecological studies listed here. AGI believes these are justified based on the low concentration of chitosan in ELEXA, its low toxicity and its widespread natural occurrence in the environment. BPB grants this request.

BPB's Conclusions and Recommendations: As a plant gowth regulator, the active ingredient has been granted an exemption from requirement of tolerance for any raw agricultural commodity (Title 40 CFR Part 180.1072), and the inert ingredient is chemically non-toxic, BPB finds the Toxicology information adequate.

DATA EVALUATION REVIEW FOR ACUTE ORAL TOXICITY (§152-10)

Product Manager: 90 **Reviewer**: Carol Glasgow, Ph.D.
MRID No.: 441858-08 **Report Date**: September 1996
Testing Laboratory: IIT Research Institute
Report No.: Project No. L08632, Study No. 1
Author(s): William D. Johnson, Ph.D., D.A.B.T.
Species: Sprague-Dawley (Crl:CD®BR) albino rat
Weight: males: 249-263 g; females: 188-219 g
Age: ~8 weeks
Sex: 5 males, 5 females
Source: Charles River Laboratories, Portage, MI
Test Material: Elexa, Lot No. AGI 02-105F; cloudy, brownish-yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

RTD/ 7/11/97

Summary:

1. **LD_{50} (mg/kg)**: >5,000

2. **Toxicity Category**: IV

3. **Classification**: Acceptable

Procedure (Deviation from §152-10): Animals acclimated minimum of 2 weeks. Rats weighed, fasted for ~19 hours and re-weighed. After fasting, rats dosed by gavage for Limit Test at 5,000 mg/kg. Feed restored approximately 3 hours later. Body weights taken additionally at days 8 and 15. Animals observed daily for 15 days and necropsied.

Results: The LD_{50} of Elexa is 5,000 mg/kg. Clinical observations in rats were normal, as were weight gains and necropsies.

BPPD's Comment: BPPD finds this material to meet the requirement for acute oral toxicity §152-10.

DATA EVALUATION REVIEW FOR ACUTE DERMAL TOXICITY (§152-11)

Product Manager: 90 **Reviewer:** Carol Glasgow, Ph.D.
MRID No.: 441858-09 **Report Date:** September 1996
Testing Laboratory: IIT Research Institute
Report No.: Project No. L08632, Study No. 2
Author(s): William D. Johnson, Ph.D., D.A.B.T.
Species: New Zealand White rabbit
Weight: males: 2.41-2.84 kg; females: 2.56-2.81 kg
Age: 12 weeks
Sex: 5 males, 5 females
Source: Kuiper Rabbit Ranch, Gary, IN
Test Material: Elexa, Lot No. AGI 02-105F; cloudy, brownish-yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

RDS 7/11/97

Summary:

1. **LD_{50} (mg/kg):** >2,000

2. **Toxicity Category:** III

3. **Classification:** Acceptable

Procedure (Deviation from §152-11): Animals acclimated 2 weeks. Test animals clipped on approximately 10% (about 240 cm^2) of the back the day prior to application and skin checked for lesions. Animals weighed immediately before dosing. Filtered test article spread on shaved dermal area and covered with a 12.8 x 11.5 cm surgical dressing. Pad covered with plastic and secured by lint-free cloth and an elastic bandage. Coverings maintained for 24 hours, wrappings removed and test sites gently wiped with a gauze pad moistened with saline and towel-dried. Animals observed for clinical signs several times after dosing and at least once daily during the observation period. Body weights taken at 7 days and termination of study. Necropsy performed on all animals.

Results: No deaths in this study. All males and 4 females gained weight appropriately, but one female gained 0.06% over the observation period. No clinical signs observed in any rabbit other than erythema in 4 males and 2 females on the first day of observation immediately after removal of coverings. Nothing unusual in necropsies.

BPPD's Comment: This study meets the requirements for §152-11, acute dermal toxicity and will be accepted by BPPD.

DATA EVALUATION REVIEW FOR ACUTE INHALATION TOXICITY (§152-12)

Product Manager: 90 **Reviewer:** Carol Glasgow, Ph.D.
MRID No.: 441858-10 **Report Date:** September 1996
Testing Laboratory: IIT Research Institute
Report No.: Project No. L08632, Study No. 6
Author(s): Stanley Vanna, B.S.
Species: Sprague-Dawley rat (Crl:CD®BR)
Weight: males: 254-275 g, females: 182-202 g
Age: ~8 weeks
Sex: 5 males, 5 females
Source: Charles River Laboratories, Portage, MI
Test Material: Elexa, Lot No. AGI 02-105F; cloudy, brownish-yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

RAS 7/11/97

Summary:
1. **LC_{50} (mg/L):** >3.6
2. **Toxicity Category:** IV
3. **Classification:** Acceptable

Procedure (Deviation from §152-12): Acclimation time for test animals at least 2 weeks. Observations were made after exposure (observations during exposure not possible due to configuration of test chamber), and daily thereafter. (No mention of excess test material being cleaned from animal fur at removal from chamber). Body weights taken prior to exposure, one week later and prior to necropsy. Necropsy performed on all animals.

Exposure conditions (recorded every 30 minutes): chamber (Cole-Parmer electronic thermohygrometer): temperature (73-74 °F), humidity (55-60 % RH); ambient: temperature (65-78 °F), humidity (61-72 % RH)

Exposure chamber: 0.5 m^3 whole body stainless steel and glass Rochester-type chamber

Particle size analysis performed on duplicate samples of material drawn from the breathing zone of the animals and measured with a Quartz Crystal Microbalance cascade impactor (California Measurements Inc.) at 23 and 85 minutes into exposure.

Concentrations measured gravimetrically. Samples taken every 60 minutes from the breathing zone of animals and collected on filters.

Aerosol generated by a Laskin nebulizer with filtered compressed air adjusted to generate appropriate aerosol.

Airflow = 103 lpm, measured every 30 minutes with a calibrated magnehelic differential pressure gauge (Dwyer Inst., Inc.) and oxygen concentration measured 7 times, with a range of 21.2-21.3% (Lynn Model 6200 oxygen analyzer).

Results: No deaths observed at the maximum achievable LC_{50} of this product for rats of $\geq$3.6 mg/L, with an MMAD ranging from 0.66 to 0.93 microns and GSD ranging from 4.15 to 3.05. All animals gained weight over the observation period. Necropsy findings unremarkable, except for 2 females having dilatated uterine horns.

BPPD's Comment: Excess material not cleaned from animals' fur after exposure as required by §81-3. Neither the MMAD nor the chamber test material concentration was continuously stable during time of measurements. The MMAD was only measured twice and increased by about 50% between the first and second reading. The chamber concentration doubled between the first and second time and increased by the same amount by the third reading. Only by the final reading (approximately the same as reading number 3) did the concentration seem to stabilize. However, BPPD will accept the data as presented for this study, §152-12.

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§152-13)

Product Manager: 90 **Reviewer:** Carol Glasgow, Ph.D.
MRID No.: 441858-11 **Report Date:** September 1996
Testing Laboratory: IIT Research Institute
Report No.: Project No. L08632, Study No. 3
Author(s): William D. Johnson, Ph.D., D.A.B.T.
Species: New Zealand White rabbit
Weight: 2.64-2.94 kg
Age: ~12 weeks
Sex: 3 male, 3 female
Source: Kuiper Rabbit Ranch, Gary, IN
Test Material: Elexa, Lot No. AGI 02-105F; cloudy, brownish-yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

BPPD 9/11/97

Summary:

1. **Toxicity Category:** IV

2. **Classification** Acceptable

Procedure (Deviation from §152-13): Animals acclimated at least 13 days before test. Eyes of rabbits examined with fluorescein and ultraviolet light prior to dosing. One-tenth milliliter of test substance instilled into conjunctival sac of right eye and eyelids held together about 2 seconds. Contralateral eye served as control. Ocular responses were recorded at one hour, and on days 1, 2 and 3 post-instillation. Fluorescein and an ultraviolet light used at 24 hours and subsequently until reversal was noted. (No mention of method used for evaluating the ocular effects, except for fluorescein.) All rabbits weighed immediately before treatment. Abnormal pharmacologic or toxic signs noted. Draize scoring system used and presented in study report.

Results: This test substance is slightly irritating to rabbit eyes. Conjunctivitis observed in 5/6 rabbits at the first reading, grade 1 for hyperemia and grade 1 for discharge in 1/6. Conjunctivitis cleared completely in all rabbits within 24 hours.

BPPD's Comment: See also marked information above on the lack of ocular observational method in the study report as required by §81.4. BPPD accepts the above study as completing the requirements for §152-13.

DATA EVALUATION REVIEW FOR PRIMARY DERMAL IRRITATION (§152-14)

Product Manager: 90 **Reviewer:** Carol Glasgow, Ph.D.
MRID No.: 441858-12 **Report Date:** September 1996
Testing Laboratory: IIT Research Institute
Report No.: Project No. L08632, Study No. 4
Author(s): William D. Johnson, Ph.D., D.A.B.T.
Species: New Zealand White rabbit
Weight: 2.57-2.89 kg
Age: ~13 weeks
Sex: 3 males, 3 females
Source: Kuiper Rabbit Ranch, Gary IN
Test Material: Elexa, Lot No. AGI 02-105F; cloudy, brownish-yellow liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

RDS 7/11/97

Summary:

1. **Toxicity Category:** IV

2.. **Classification:** Acceptable

Procedure (Deviation from §152-14): Animals acclimated for 3 weeks. Rabbits shaved on dorsal area of trunk (~240 cm^2) and skin examined for irritation on day prior to test. Filtered test material (0.5 ml) applied to a test site (not defined) and covered with a 2.5 x 2.5 cm 12-ply cotton gauze pad secured with porous tape. Rabbits were wrapped in lint-free cloths secured by elastic adhesive bandages (Elastoplast®). After 4 hours exposure, wrappings removed and the test site rinsed with water and wiped with gauze. Rabbits weighed immediately before dosing. Observations for erythema and edema were made at 1, 24, 48 and 72 hours following patch removal. Draize grading scale used for scoring.

Results: This product is not a dermal irritant. No rabbit exhibited any erythema or edema at any point in the study.

BPPD's Comment: Acceptable to BPPD to complete the requirements for §152-14.

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DATA EVALUATION REVIEW FOR DERMAL SENSITIZATION (§152-15)

Product Manager: 90 **Reviewer**: Carol Glasgow, Ph.D.
MRID No.: 441858-13 **Report Date**: September 1996
Testing Laboratory: IIT Research Institute
Report No.: Project No. L08632, Study No. 5
Author(s): William D. Johnson, Ph.D., D.A.B.T.
Species: Hartley albino guinea pig
Weight: 345 - 449 g
Age: ~5½ weeks
Sex: 30 males
Source: Sasco, Inc., Madison, WI
Test Material: Elexa, Lot No. AGI 02-105F; cloudy, brownish-yellow liquid
Positive Control: 1-chloro-2,4-dinitrobenzene (DNCB): induction - 0.3 ml of 0.05% DNCB w/v in 95% ethanol; challenge - same as induction
Quality Assurance (40 CFR §160.12): Included, acceptable

RPD 7/11/97

Method: Modified Buehler

Summary: 1. **Rating**: Non-sensitizer

2. **Classification**: Acceptable

Procedure (Deviation from §152-15): Acclimation period ~2 weeks. (No irritation screening of test substance performed.) Both the induction concentration and the challenge were 100%. During induction, 10 pre-weighed animals treated with 0.3 ml test substance once each week for 3 weeks on pre-clipped test sites under an occlusive 25 mm Hilltop Chamber secured with elastic adhesive bandage (Elastoplast®). After 6 hours of exposure, chambers removed. Ten pre-weighed animals used for sham control study and treated identically to test material animals, but with no test material applied. Two weeks following the last induction treatment, challenge dose applied to test animals and naive controls. Skin evaluated 24 and 48 hours after chamber removal. Readings repeated after first induction application, and after challenge. All guinea pigs depilated with Neet® hair remover approximately 24 hours prior to 24 hour scoring for challenge phase. Animals weighed weekly. Draize scale used for evaluation scores. (Positive control animals (10 pre-weighed) treated in the same manner, but no sham control group included.)

Results: No dermal sensitization exhibited with this test substance. All animals gained weight and none exhibited toxic reactions. 100% of the positive controls were significantly positive.

BPPD's Comment: Several minor comments are given in the parentheses listed above in the **Procedure** section. However, BPPD accepts the results as indicating non-sensitization and completing the requirements for §152-15.

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